

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/137378/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Swann, Karl ORCID: <https://orcid.org/0000-0002-4355-1449> 2020. The soluble sperm factor that activates the egg: PLCzeta and beyond. Reproduction 160 (1) 10.1530/REP-20-0079 file

Publishers page: <http://dx.doi.org/10.1530/REP-20-0079>
<<http://dx.doi.org/10.1530/REP-20-0079>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies.

See

<http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



The soluble sperm factor that activates the egg: PLCzeta and beyond.

Karl Swann

School of Biosciences

Cardiff University.

The Sir Martin Evans Building

Museum Avenue

Cardiff CF10 3AX

Swannk1@cardiff.ac.uk

Short title: PLC ζ and beyond.

Key words: sperm, egg, fertilization, calcium, PLC ζ

Word count: 1739

Abstract

PLCzeta(ζ) initiates Ca^{2+} oscillations and egg activation at fertilization in mammals, but studies in mouse eggs fertilized by PLC ζ knockout (KO) sperm imply that there is another slow acting factor causing Ca^{2+} release. Here, I propose a hypothesis for how this second sperm factor might cause Ca^{2+} oscillations in mouse eggs.

Egg activation is caused by increases in cytosolic Ca^{2+} , and in mammalian eggs (MII oocytes) the sperm triggers a prolonged series of repetitive transients, or oscillations, in the cytosolic free Ca^{2+} concentration (Swann & Lai 2016, Sanders & Swann 2016). These Ca^{2+} oscillations are driven by increased inositol 1,4,5-trisphosphate (InsP_3) production which causes cycles of Ca^{2+} release from the InsP_3 -receptor (IP_3R). Since the 1990s we have known that mammalian sperm contain a soluble protein 'sperm factor' (or sperm-oocyte-activating-factor- SOAF), that can trigger Ca^{2+} oscillations after gamete fusion (Swann and Lai, 2016). Its existence inside the sperm can explain why intracytoplasmic sperm injection (ICSI) mimics fertilization in causing Ca^{2+} oscillations in mouse and human eggs (Jones 2018, Kurokawa & Fissore 2003). It is now widely acknowledged that this sperm factor in mammals is the sperm-specific protein phospholipase PLCzeta(ζ) (Swann & Lai 2016, Jones 2018). Key evidence includes the finding that microinjection of PLC ζ cRNA or protein causes prolonged sperm-like Ca^{2+} oscillations in all mammalian eggs studied (Swann & Lai 2016), and that functionally disruptive mutations in PLC ζ alone lead to male factor infertility and egg activation failure in humans in IVF and ICSI (Escoffier *et al.* 2016).

Recently two groups have reported the phenotype of PLC ζ knock out (KO) mice. They both found that injecting PLC ζ KO mouse sperm into eggs (hence ICSI) fails to trigger any Ca^{2+} oscillations (Hachem *et al.* 2017, Nozawa *et al.* 2018). This shows that PLC ζ accounts for the Ca^{2+} signals and egg activation after ICSI. However, during *in vitro* fertilization (IVF) and mating with PLC ζ KO males some eggs are activated at fertilization and embryo development still occurs (Hachem *et al.* 2017, Nozawa *et al.* 2018). Success rates of IVF are lower and litter sizes are smaller with PLC ζ KO males but the result contrasts with what happens with ICSI. The reason why IVF leads some

eggs to activate with PLC ζ KO sperm is because there are ~3 large Ca²⁺ oscillations that occur about 40 mins later than expected when compared to wild type sperm (Nozawa *et al.* 2018). The existence of these delayed Ca²⁺ oscillations with PLC ζ KO sperm has been reproduced in my lab (Fluks, Parrington and Swann unpublished). The late Ca²⁺ oscillations with PLC ζ KO sperm lead to delayed egg activation, including cortical granule exocytosis which is required to block extra sperm entry (Nozawa *et al.* 2018). This means that many such zygotes fail to develop because they are polyspermic. Overall, the data suggest that PLC ζ initiates the Ca²⁺ oscillations at fertilization, accounting for most of the Ca²⁺ spikes, but that during IVF the sperm has another mechanism for promoting later Ca²⁺ oscillations in the mouse (Jones 2018). Two characteristics of this secondary mechanism is that it is delayed after gamete fusion, and that it is active in IVF and not with ICSI.

In looking for PLC ζ -independent mechanisms for Ca²⁺ oscillations we need to consider previous data gathered from mammalian zygotes. First, all previous studies have shown that without sperm-egg membrane fusion in IVF there are no Ca²⁺ oscillations (Swann & Lai 2016). So, it's reasonable to assume that a second mechanism for Ca²⁺ release involves a sperm factor that is either soluble and enters the egg by cytosolic diffusion, or that it is introduced by the sperm membrane into the egg plasma membrane by two-dimensional diffusion. For either option I will describe it as a sperm factor. It has been suggested that the PLC ζ -independent sperm factor may be 'cryptic' because it is only apparent when PLC ζ is absent (Jones 2018). Whilst this is true from an observational point of view, it does not mean it is inactive during normal fertilization. In fact, it is difficult to see how a second factor could only arise when PLC ζ was not present. As far as we know PLC ζ is only active in eggs, so a lack of PLC ζ would not be evident until after gametes have fused. Clearly, gene expression in spermatogenesis cannot compensate for future events, hence the second factor should operate in IVF with wild type sperm. In hindsight we can see evidence of a secondary mechanism because it was previously found that ICSI causes a shorter duration of Ca²⁺ oscillations than IVF in mouse zygotes⁵. If the secondary factor operates in normal IVF it also gives it a selective advantage for it to persist in the presence of PLC ζ . One attractive idea is that this

72 factor is a 'primitive' factor from a role in egg activation in species earlier in the vertebrate lineage
 73 (Nozawa *et al.* 2018).
 74
 75 Previous studies restrict the options for how any factor can trigger Ca^{2+} oscillations in the absence
 76 of PLC ζ . For example, one could propose that the second factor promotes Ca^{2+} influx into the egg,
 77 perhaps by the insertion of sperm derived Ca^{2+} channels into the egg membrane. However, there
 78 are many ways to increase Ca^{2+} influx into unfertilized mammalian eggs and none of them trigger
 79 Ca^{2+} oscillations without PLC ζ . An updated version of the ' Ca^{2+} conduit' idea remains implausible
 80 (Swann & Lai 2016). The second factor cannot work via messengers such as NAADP, or cADPR,
 81 since these also fail to trigger Ca^{2+} oscillations in mouse eggs (Swann & Lai 2016). A sperm protein
 82 called PAWP has been suggested to trigger Ca^{2+} oscillations in eggs, but the key data on PAWP is
 83 not reproducible (Sanders and Swann, 2016). Furthermore, PAWP is supposed to cause Ca^{2+}
 84 oscillations during ICSI, but we now know that PLC ζ accounts for these Ca^{2+} oscillations. Another
 85 study has suggested that extramitochondrial citrate synthase is the second sperm factor in
 86 mammals (Kang *et al.* 2020). However, the phenotype of extramitochondrial citrate synthase KO
 87 sperm at fertilization is apparently the same as PLC ζ KO sperm, with delayed Ca^{2+} oscillations
 88 (Kang *et al.* 2020). This result is difficult to rationalize because these citrate synthase KO sperm
 89 still contained PLC ζ and the initial Ca^{2+} oscillations should not be delayed. In addition, we have
 90 found that citrate synthase protein injection into mouse eggs does not trigger Ca^{2+} release
 91 (Sanders and Swann, unpublished observations). From what we know about how to cause Ca^{2+}
 92 oscillations in mouse eggs, we can conclude that the second factor is either making InsP_3 , or else
 93 directly stimulating the IP_3Rs .
 94
 95 If the second sperm factor generates InsP_3 this implicates another PLC. There are many other PLC
 96 isoforms in mammalian sperm (Parrington *et al.* 2002). However, the other PLCs are about two or
 97 three orders of magnitude less active in causing Ca^{2+} release than PLC ζ in eggs (Swann and Lai
 98 2016; Mehlmann *et al.* 2001). To be active in eggs they would have to be expressed at >2pg per
 99 sperm, and yet there is only 40pg of total protein in a mouse sperm (Mehlmann *et al.* 2001). The

100 second sperm factor could stimulate an egg membrane derived PLC, but this is not consistent with
101 some previous findings. For example, if eggs are imaged using GFP tagged C1 domains, there is
102 no measurable diacylglycerol increase in the plasma membrane for at least two hours of sperm
103 induced Ca^{2+} oscillations in mouse eggs, despite the ability of this probe to respond to other stimuli
104 (Yu *et al.* 2008). Hence, it appears that a plasma membrane derived PLC activity is not stimulated
105 in fertilizing eggs. This is not an issue for $\text{PLC}\zeta$ which is the only mammalian PLC without a PH
106 domain and it binds to PIP_2 in intracellular vesicles (Fig 1) and not the plasma membrane (Swann
107 & Lai 2016). However, conventional PLCs (β , γ or $\delta 1$ class) locate to the plasma membrane with a
108 PH domain, and one would expect some diacylglycerol increase to occur if they were active at
109 fertilization. If the second sperm factor makes InsP_3 then it probably needs to stimulate another
110 unconventional PLC that is localized on vesicles in the egg. It is not clear whether any other PLCs
111 would match the unusual localization pattern of $\text{PLC}\zeta$, but it might be worth investigating the
112 localization of the epsilon or eta class of PLCs in eggs.

113
114 In the absence of data on other PLCs I can suggest an alternative idea, that the second sperm
115 factor acts to sensitize the IP_3R . Strontium ions or thimerosal both stimulate Ca^{2+} oscillations in
116 mouse eggs, and they both appear to act directly to sensitize the IP_3R to release Ca^{2+} (Swann &
117 Lai 2016). The schematic in Fig.1 shows the second factor affecting IP_3R induced Ca^{2+} release
118 following $\text{PLC}\zeta$ entry. If the target is the IP_3R , or vesicular PIP_2 , the protein factor is likely to be
119 soluble and diffuse into the cytosol. To explain why $\text{PLC}\zeta$ independent Ca^{2+} release is not evident
120 with ICSI, it is possible that the second factor is released from the sperm during their preparation
121 when the sperm is damaged, or when the head is removed. Damaging the sperm membrane is
122 standard practice before ICSI. Plasma membrane damage during cryopreservation may also lead
123 to the loss of the second factor from sperm, which could explain why there was a lack of Ca^{2+}
124 oscillations with most cryopreserved $\text{PLC}\zeta$ KO sperm in IVF (Hachem *et al.* 2017). The other
125 feature of the second sperm factor is a delayed action. It could be that the synthesis of a second
126 sperm factor protein from RNA in the sperm could account for the >40 min delay before Ca^{2+}
127 transients (Jones 2018). However, the total amount of RNA in a single mouse sperm (0.1pg) is

128 similar to the amount of PLC ζ RNA injected into an egg to cause Ca²⁺ oscillations, and yet sperm
129 RNA is made up of several hundred varieties. Any protein synthesized from sperm RNA would
130 have to be >100 times more potent than PLC ζ which is active at concentrations of less than 10nM.
131 A more realistic idea is that secondary factor is another protein delivered by the sperm. The delay
132 could be because this protein needs to first diffuse and equilibrate throughout the egg and then act
133 indirectly to sensitize IP₃Rs. The second factor may not be active in human fertilization since
134 human eggs are less sensitive to Ca²⁺ release, and for example do not oscillate in response to
135 strontium medium (Lu *et al.* 2018). This could explain why inactivating mutations in human
136 PLC ζ lead to male factor infertility with both normal conception and ICSI (Escoffier *et al.* 2016). The
137 second factor may only be evident in mouse and rat eggs, or possibly in some non-mammalian
138 species that do not appear to use PLC ζ to activate the egg (Swann & Lai 2016).

139

140

141 **Declaration of Interests**

142 The author declares that there is no conflict of interest that could be perceived as prejudicing the
143 impartiality of this article.

144 **Funding.**

145 This research did not receive any specific grant from any funding agency in the public, commercial
146 or not-for-profit sector.

147 **Author Contribution.**

148 KS conceived the ideas and wrote the paper.

149 **Acknowledgements.**

150 I am grateful to Jessica Sanders and Monika Fluks who helped generate the unpublished data I
151 refer to, and to John Parrington for sending me the PLC ζ KO male mice.

152

153 **References**

154

Escoffier J, Lee HC, Yassine S, Zougari R, Martinez G, Karaouzene T, Coutton C, Kherraf ZE, Halouni L, Triki C, Nef S, Thierry-Mieg N, Savinov SN, Fissore R, Ray PF & Arnoult C (2016) Homozygous mutation of PLC ζ 1 leads to defective human oocyte activation and infertility that is not rescued by the WW-binding protein PAWP, Hum. Mol. Genet. **25** 878-891. (<https://doi.org/10.1093/hmg/ddv617>)

Hachem A, Godwin J, Ruas M, Lee HC, Ferrer-Buitrago M, Ardestani G, Bassett A, Fox A, Naverrete F, De Sutter P, Heindryckx B, Fissore RA & Parrington J (2017) PLC ζ is the physiological trigger of the Ca²⁺ oscillations that induced embryogenesis in mammals but conception can occur in its absence, Development. **144** 2914-2924. (<https://doi.org/10.1242/dev.150227>)

Jones KT (2018) Mammalian sperm contain two factors for calcium release and egg activation: Phospholipase C zeta and a cryptic activating factor. Molecular Human Reproduction. **24** 465-468. (<https://doi.org/10.1093/molehr/gay038>)

Kang W, Harada Y, Yamatoya K, Kawano N, Kanai S, Miyamoto Y, Nakamura A, Miyado M, Hayashi Y, Kuoki Y, Saito H, Iwao Y, Umezawa A & Miyado K (2020) Extra-mitochondrial citrate synthase initiates calcium oscillation and suppresses age-dependent sperm dysfunction. Laboratory Investigation. **100**, 583-595 (<https://doi.org/10.1038/s41374-019-0353-3>)

Kurokawa M & Fissore RA (2003) ICSI-generated mouse zygotes exhibit altered calcium oscillations, inositol 1,4,5-trisphosphate receptor 1 downregulation, and embryo development. Molecular Human Reproduction **9**, 523-533. (<https://doi.org/10.1093/molehr/gag072>)

Lu Y, Reffy R, Ferrer Buitrago M, Vander Jeught M, Neupane J, De Vos WH, Van der Abbeel E, Lierman S, De Sutter P & Heindryckx B (2018) Strontium fails to trigger Ca²⁺ release and activation in human oocytes despite the presence of functional TRPV3 channels. Human Reproduction Open **3**, hoy005. (<https://doi.org/10.1093/hropen/hoy005>)

Mehlmann LM, Chattopadhyay A, Carpenter G and Jaffe LA (2001) Evidence that phospholipase C from the sperm is not responsible for initiating Ca^{2+} release at fertilization in mouse eggs. *Dev. Biol.* **236**, 492-501. (<https://doi.org/10.1006/dbio.2001.0329>)

Nozawa K, Satouh Y, Fujimoto T, Oji A, & Ikawa M (2018) Sperm borne phospholipase C zeta1 ensures monospermic fertilization in mice. *Sci. Rep.* **8**, 1315. (<https://doi.org/10.1038/s41598-018-19497-6>)

Parrington J, Jones ML, Tunwell R, Devader C, Katan M & Swann K (2002) Phospholipase C isoforms in mammalian spermatozoa: potential components of the sperm factor that causes Ca^{2+} release in eggs. *Reproduction* **123**, 31-39. (<https://doi.org/10.1530/rep.0.1230031>)

Sanders J & Swann K (2016) Molecular triggers of egg activation at fertilization in mammals, *Reproduction* **152**, R41-R50. (<http://dx.doi.org/10.1530/REP-16-0123>)

Swann K & Lai FA (2016) Egg activation by a soluble sperm protein. *Physiological Reviews* **96**, 127-149. (<https://doi.org/10.1152/physrev.00012.2015>)

Yu Y, Halet G, Lai FA & Swann K (2008) Regulation of diacylglycerol production and protein kinase C stimulation during sperm- and $\text{PLC}\zeta$ -mediated mouse egg activation. *Biol. Cell* **100**, 633-643. (<https://doi.org/10.1042/BC20080033>)

204 **Figure Legend.**

205

206 Fig.1. A schematic representation of the hypothesis for PLC ζ and a second factor may act to
207 cause Ca²⁺ oscillations in fertilizing mouse eggs.

